## REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 38-44 and 51 have been amended, and new claims 52-59 have been added. New claims 52-59 find descriptive support in the claims as filed, as well as the disclosure of SEQ ID NOS:1-8 and fragments thereof (pp. 12-25 of specification).

The rejection of claims 38-44 and 46-51 under 35 U.S.C. § 112, first paragraph, as lacking written descriptive support for the claimed genus is respectfully traversed.

The burden of establishing that an application lacks adequate written descriptive support falls on the U.S. Patent and Trademark Office ("PTO"). *See In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). Hence, the PTO must demonstrate *why* the disclosure is insufficient.

The PTO has taken the position at pages 3-6 of the outstanding office action that the four exemplary nucleic acids (SEQ ID NO: 2 encoding HrpN of *Erwinia chrysanthemi*, SEQ ID NO: 4 encoding HrpN of *Erwinia amylovora*, SEQ ID NO: 6 encoding HrpZ of *Pseudomonas syringae*, and SEQ ID NO: 8 encoding PopA1 of *Pseudomonas solanacearum*) are not representative of the claimed genus. The basis for the PTO's position is that a number of pathogen species—in the sense of biological classification—exist, and hypersensitive response elicitors from only several of the many pathogen species have been described in the specification. Applicants submit that this basis for asserting lack of written descriptive support is insufficient.

The Federal Circuit has clearly espoused that *per se* conclusions of written description violations cannot be founded upon the basis of genus size alone. *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316, 1326-27, 63 USPQ2d 1609, 1614-15 (Fed. Cir. 2002) (refusing to adopt position that three species as a matter of law cannot satisfy written description requirement for significantly larger genus). Thus, the PTO's conclusion cannot be based on genus size alone. But that is precisely what the PTO has done at pages 3-6 of the outstanding office action. The PTO lists numerous organisms, suggests that the majority of these organisms would produce at least one hypersensitive response elicitor, and then concludes that neither the claimed genus nor the subgenera from any bacteria are

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adequately described. Because the PTO's position is unsupported by law and unsupported by any facts other than genus size, applicants submit that the PTO's position cannot be sustained.

With respect to the PTO's citation of several hypersensitive response elicitors identified after the priority filing date of the present application (e.g., HrpW of *Erwinia amylovora* and *Pseudomonas syringae*), it should be noted that the specification teaches those of skill in the art that these elicitors, too, can be used to practice the invention even though their nucleotide and amino acid sequences are not recited specifically in the specification. It should be noted that the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶ 1, 'Written Description' Requirement," make explicitly clear that the description of a representative number of species does *not* require the description to be of such a nature that it would provide support for each species that the genus embraces. 66 Fed. Reg. 1099, 1106 (2001). Hence, the absence of sequences for the later-identified HrpW elicitors is irrelevant to the issue of whether the present specification provides adequate written descriptive support for their use in accordance with the present invention.

Moreover, the conclusion by the PTO is contrary to evidence previously submitted by applicants in this case. As demonstrated in the Declaration of Zhong-Min Wei Under 37 C.F.R. § 1.132 (dated September 25, 2002) ("Wei Declaration") and the Supplemental Declaration of Zhong-Min Wei Under 37 C.F.R. § 1.132 (dated January 21, 2003) ("Supplemental Wei Declaration"), one of ordinary skill in the art would have understood that applicants were in possession of the presently claimed invention at the time the present application was filed. This is so, because the four exemplary species were recognized at the time of filing as belonging to an art-recognized class of hypersensitive response eliciting proteins produced by bacterial plant pathogens.

One reason why the disclosed species are representative of the claimed genus is because the species were recognized as structurally and functionally conserved. For example, it was known that hypersensitive response elicitors within a given genus—again, in the sense of biological classification—are often homologous to elicitors from different pathogenic species and strains of the same genus. *See* Supplemental Wei Declaration ¶ 6. This has been demonstrated among HrpN homologs from *Erwinia*, where the *Erwinia amylovora hrpN* gene has been used to clone other *hrpN* homologs from different *Erwinia* species (*see* Supplemental Wei Declaration ¶¶ 7-9); and HrpZ homologs from *Pseudomonas*, where the *Pseudomonas syringae hrpZ* gene has been used to clone other *hrpZ* homologs from different *Pseudomonas syringae* pathovars (*see* Supplemental Wei Declaration ¶ 10). Further, the genes encoding the HrpN hypersensitive response elicitor from several strains of

*Erwinia pyrifolia* have since been cloned. *See* Jock et al., "Molecular Differentiation of *Erwinia amylovora* Strains from North America and of Two Asian Pear Pathogens by Analyses of PFGE Patterns and *hrpN* genes," *Environ. Microbiol.* 6(5): 480-490 (2004) ("Jock") (attached hereto as **Exhibit 1**). As reported in Jock, the *hrpN* genes were amplified with PCR consensus primers that were deduced by comparison of the known nucleotide sequences of *Erwinia amylovora hrpN* and *Erwinia stewartii hrpN*. Indeed, Jock (at page 489) recites the following:

> *Erwinia pyrifoliae* and the *Erwinia* strains from Japan were considered to be sufficiently related to *E. amylovora* to amplify their genes with the *Erwinia* PCR consensus primers given above. This was indeed possible and allowed cloning and sequencing of their *hrpN* DNA fragments. . . .

In view of the above, one of ordinary skill in the art would expect structural conservation of hypersensitive response elicitors, at least among the pathogens classified as belonging to the same genus (again, in the sense of biological classification).

Another reason why the disclosed species are representative of the claimed genus is because the encoding genes are similarly regulated, expressed, and secreted by their source organisms. For instance, the genes encoding hypersensitive response elicitors are positioned within the *hrp* gene cluster or proximate to the *hrp* gene cluster in *hrp* regulons. *See* Supplemental Wei Declaration ¶ 11. Substantially all hypersensitive response elicitors identified have been shown to be secreted through the type III (or *hrp*-dependent) secretion pathway, which is a highly conserved and unique mechanism for the delivery of pathogenicity related molecules in gram-negative bacteria. *See* Supplemental Wei Declaration ¶ 13. Finally, expression of the genes encoding the *hrp* gene cluster is induced under conditions that mimic the plant apoplast, such as low concentrations of carbon and nitrogen, low temperature, and low pH. *See* Supplemental Wei Declaration ¶ 14. Thus, because the encoding genes are similarly regulated, expressed, and secreted by their source organisms, one of ordinary skill in the art would expect other hypersensitive response elicitor genes to behave similarly.

Another reason why the disclosed species are representative of the claimed genus is because the disclosed species are characterized by a number of common biochemical characteristics which were known to those of skill in the art prior to the filing date of the present application. These include being glycine rich, heat stable, hydrophilic, lacking of an N-terminal signal sequence, and susceptible to proteolysis. *See* Supplemental Wei Declaration ¶ 15.

A final reason why the disclosed species are representative of the claimed genus is because these species share the ability to induce specific plant responses. The induction of plant disease resistance, plant growth enhancement, and plant stress resistance are three plant responses that result from treatment of plants or plant seeds with a hypersensitive response elicitor from a bacterial plant pathogen. *See* Supplemental Wei Declaration ¶ 17.

With respect to growth enhancement, both topical application and transgenic expression have proven to be useful. Topical application of hypersensitive response elicitors from a range of sources, such as *Pseudomonas syringae* (HrpZ) and *Xanthomonas campestris* (HreX), was shown to enhance the growth of tomato plants. *See* Wei Declaration ¶¶ 4 and 9-11. Growth enhancement was also shown to be induced by transforming cotton and *Arabidopsis* plants with a DNA molecule encoding the HrpN hypersensitive response elicitor from *Erwinia amylovora*. *Id.* at ¶¶ 4-8. In view of the above-described data showing induction of plant growth enhancement by different hypersensitive response elicitors from multiple bacterial sources, including those disclosed by sequence and those not disclosed by sequence in the present application, one of ordinary skill in the art would expect other members of this art-recognized class to likewise induce growth enhancement in plants following topical application or transgenic expression of such bacterial hypersensitive response elicitors.

As described above and as supported by the Wei Declaration and the Supplemental Wei Declaration, applicants have presented a body of evidence demonstrating that the four species belong to an art-recognized class of proteins from bacterial plant pathogens; that structural conservation exists among homologs of the different hypersensitive response elicitor proteins; that hypersensitive response elicitor genes are similarly regulated, expressed, and secreted by type III secretion systems of their source organisms; that the hypersensitive response elicitor proteins are characterized by a number of common biochemical characteristics which were known to those of skill in the art prior to the filing date of the present application; and that the hypersensitive response elicitor proteins of this art-recognized class are functionally similar in their ability to induce similar plant responses, particularly growth enhancement. The PTO, on the other hand, has merely suggested that the genus is large and may contain many species—though the PTO did not demonstrate that the genus contains structurally and functionally unrelated species.

For all these reasons, the rejection of claims 38-44 and 46-51 as lacking written descriptive support and should be withdrawn.

The rejection of claims 38-44 and 46-51 under 35 U.S.C. § 112, first paragraph, for lack of enablement is respectfully traversed.

The rejection is premised on the asserted failure to teach one of ordinary skill in the art how to make and/or use the invention with regard to species of nucleic acids (encoding hypersensitive response elicitors) that are not described by nucleotide sequence in the present application. For substantially the same reasons noted above, applicants submit that this rejection is improper.

All that is needed is objective enablement of what is claimed. *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). That is precisely what the specification provides, when the disclosure is considered with the state of the art at the time the application was filed. One of ordinary skill in the art is fully capable of identifying other hypersensitive response elicitor-encoding nucleic acids (*see* Supplemental Wei Declaration ¶¶ 6-10), determining whether the encoded protein does, in fact, induce a hypersensitive response in non-host plants (*see* Supplemental Wei Declaration ¶ 5), preparing DNA constructs and transforming plants as described in the specification at page 32, line 28 to page 33, line 18, and then ascertaining whether the transformed plants exhibit enhanced growth (*see* Examples 1-24; *see also* Wei Declaration ¶¶ 7-11) (describing methods of testing for growth enhancement in a variety of plants). Thus, the teachings of the present application, coupled with the knowledge in the art, would have allowed one of skill in the art to practice the claimed invention within the full scope of the claims, even with nucleic acids not explicitly disclosed therein.

For these reasons, the rejection of claims 38-44 and 46-51 for lack of enablement is improper and should be withdrawn.

The rejection of claims 39-44 and 51 under 35 U.S.C. § 112, second paragraph, for indefiniteness is respectfully traversed in view of the above amendments.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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# Molecular differentiation of *Erwinia amylovora* strains from North America and of two Asian pear pathogens by analyses of PFGE patterns and *hrpN* genes

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## Summary

**In order to determine a possible genomic divergence of *Erwinia amylovora* 'fruit tree' and raspberry strains from North America, several isolates were differentiated by pulsed-field gel electrophoresis (PFGE) analysis, the size of short DNA sequence repeats (SSRs) and the nucleotide and deduced amino acid sequences of their *hrpN* genes. By PFGE analysis European strains are highly related, whereas strains from North America were diverse and were further distinguished by the SSR numbers from plasmid pEA29. The *E. amylovora* strains from Europe showed identical HrpN sequences in contrast to the American isolates from fruit trees and raspberry. Those were related to each other, but distinguishable by their HrpN patterns. The Asian pear pathogens differed in HrpN among each other and from *E. amylovora*. *Erwinia pyrifoliae* isolates and the *Erwinia* strains from Japan were ordered via their HrpN sequences in agreement with the PFGE patterns. For all three pathogens, dendrograms from PFGE and sequence data indicate an evolutionary diversity within the species in spite of a genetic conservation for parts of the *hrpN* genes suggesting a long persistence of the Asian pear pathogens in Korea and Japan as well as of fire blight in North America. Some of the divergent American *E. amylovora* isolates share PFGE patterns with the relatively uniform European strains.**

## Introduction

Fire blight of apple and pear fruit trees and raspberry as well as of other rosaceaous plants is assumed to have originated in the Eastern part of North America, from where the disease might have been endemic for a long time, and was then distributed in the last century to many countries of the Northern hemisphere and to New Zealand (Bonn and van der Zwet, 2000). In Korea, a bacterial disease of pears and its causative agent *Erwinia pyrifoliae* has been described (Rhim *et al.*, 1999), which was distinguished from *Erwinia amylovora* by molecular and microbiological tools (Kim *et al.*, 1999) and additional DNA sequences (McGhee *et al.*, 2002). Another disease, bacterial shoot blight of pear, was noticed on the island of Hokkaido in Japan (Beer *et al.*, 1996) and the pathogen has been shown to be more related to *E. pyrifoliae* than to *E. amylovora* (Kim *et al.*, 2001a).

*Erwinia amylovora* has been extensively investigated for many physiological, biochemical and molecular features (reviewed in Vanneste, 2000). Two main factors are a strict requirement for pathogenicity: the ability to produce the acidic exopolysaccharide (EPS) amylovoran, encoded in the 17 kb *ams* region of the chromosome (Bugert and Geider, 1995) and to induce a hypersensitive response (HR) on non-host plants, encoded by the 30 kb *hrp* region (Kim and Beer, 2000). The large number of *hrp* genes is associated with regulation and transport of two elicitor proteins, HrpN (harpin) (Wei *et al.*, 1992) and HrpW (Barny, 1995). The adjacent *dsp* region with *dspA/E* (Gaudriault *et al.*, 1997; Bogdanove *et al.*, 1998) may contribute to harpin activity. Because mutagenesis of *hrpN* revealed residual HR-inducing activity of HrpN-fragments, HrpN might not be strictly required as an intact protein (Barny, 1995) and conservation of its sequence has not been strongly selected in mutational changes during evolution. Accordingly, its DNA and amino acid sequences could be open to changes without affecting bacterial fitness and may be useful for strain and species differentiation.

Another molecular tool for differentiation of *E. amylovora* and *E. pyrifoliae* as well as the *Erwinia* strains from Japan is PFGE analysis (Zhang and Geider, 1997; Zhang *et al.*, 1998; Jock *et al.*, 2002). Macrorestriction of the bacterial genome revealed several closely related but distinguishable pattern types for *E. amylovora* which were used to follow spread of fire blight in Europe and in the Mediterranean region (Jock *et al.*, 2002). Another method to distinguish *E. amylovora* strains and the *Erwinia* strains

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from Japan was determination of short sequence DNA repeats (SSR) in the PCR fragment amplified with primers P29A and P29B from the common *E. amylovora* plasmid pEA29 (Kim and Geider, 1999), also applied to the *Erwinia* strains from Japan (Jock *et al.*, 2003a). In contrast to *E. amylovora* strains from Europe and the Mediterranean region, heterogeneous PFGE patterns of American strains could indicate a long persistence of the pathogen in North America. Based on HrpN-sequences, *E. pyrifoliae* strains from Korea (Kim *et al.*, 2001b) and pear-pathogenic *Erwinia* strains from Japan (Kim *et al.*, 2001a) were also divergent. Accordingly, macrorestriction and *hrpN* sequence analysis can be used for differentiation and grouping of strains within the three pathogens.

## Results

### *PFGE patterns of* E. amylovora *strains isolated in North America from fruit trees and raspberry*

To estimate possible diversity of *Erwinia amylovora* strains in North America, we collected a set of strains in several areas of Eastern Canada. The samples were derived from fire blight-infected orchards with pear and apple trees located in Nova Scotia near Kentville and in Ontario near Toronto as well as from hawthorn adjacent to the apple orchard in the Kentville area. After an *Xba*I digest (Fig. 1A, Table 1), the strains isolated from hawthorn and apple trees from Kentville carry the PFGE pattern Pt4 as found before (Jock *et al.*, 2002) for strains isolated in England, Western France and Northern Spain. Strikingly, the strains from pears which are isolated in Nova Scotia in an orchard only 100 km apart from the apple orchard, had a different pattern. Another divergent pattern type was found for strains isolated in pear orchards of the Ontario region. The divergence or similarity of the investigated isolates can be deduced from the dendrogram in Fig. 1B.

An additional set of strains was isolated in Eastern Canada 1997 in the Kentville area of Nova Scotia. Strains from apple trees had the same pattern as the strains from hawthorn and apple isolated in 2000 (Table 1). Some shared the PFGE pattern with the European pattern types Pt1, others with Pt4. Most others were quite divergent in contrast to the closely related European pattern types.

Remarkably, *E. amylovora* strains isolated in Europe and in the Mediterranean region have an identical PFGE pattern in an *Spe*I digest except for one band shifted for strains of the *Xba*I pattern type Pt3 (Zhang and Geider, 1997). In contrast, the strains from America were divergent in their *Spe*I pattern (Fig. 2A), except strains EaCa4/97 and EaCa6/97 with an identical *Spe*I pattern, which were isolated in the same year and area. Three strains which were isolated in Eastern Canada from raspberry, an alternative host for fire blight, differed in their PFGE

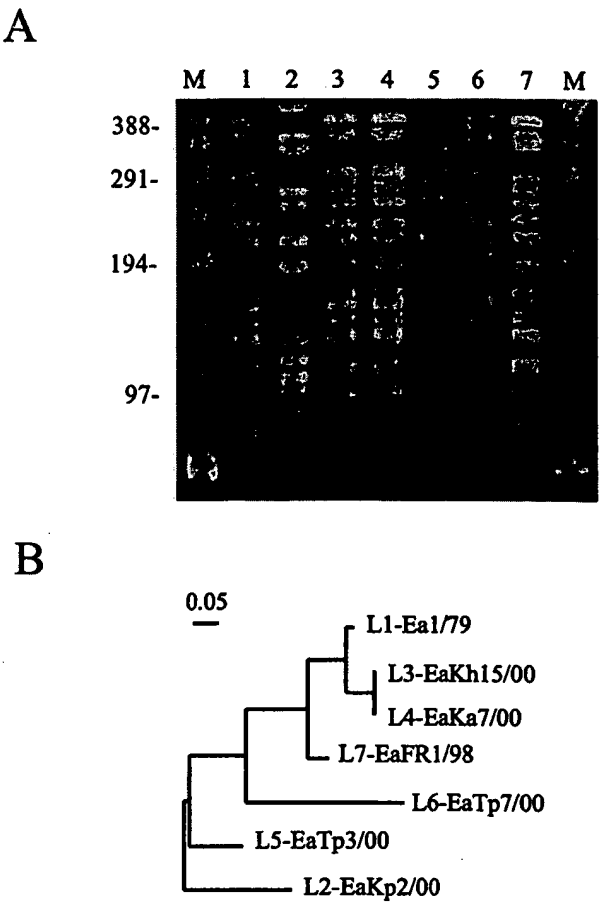


**Fig. 1.** PFGE analysis of *E. amylovora* strains isolated in Canada after genomic *Xba*I digests.
A. Lanes: M: λ DNA marker (sizes at left in kb); 1: Ea1/79 (Pt1, standard pattern for central Europe); 2: EaKp2/00; 3:EaKh15/00; 4: EaKa7/00; 5: EaTp3/00; 6: EaTp7/00 (Isolates from Eastern Canada.); 7: EaFR1/98 (from Germany); *Xba*I digests.
B. Dendrogram from patterns in A. Bar, distance scale.

patterns after *Xba*I and *Spe*I digests among each other and showed barely overlapping patterns with 'fruit tree' strains (Fig. 2A, Table 1). The raspberry strain IL6 from Illinois is more related to the 'fruit tree' strain Ea1/79 than the other rubus strains assayed.

### *The sizes of SSRs of strains from a narrow region of Eastern Canada*

A more variable feature than PFGE patterns of the *E. amylovora* genome is a DNA fragment from the common plasmid pEA29 with several short sequence DNA repeats (Kim and Geider, 1999; Jock *et al.*, 2003a). The SSR numbers are not related to the PFGE patterns, enabling differentiation of strains with the same pattern by SSR numbers. Rarely, the SSR numbers differ for strains isolated from plants in the same region. Nevertheless, strains

**Table 1.** Bacteria used in the experiments.

| Strain | Description of isolation (plant, place, year, provider) | PFGE pattern[a] |
|---|---|---|
| *E. amylovora* strains from Canada (fruit tree) | | |
| EaCa1/00 | pear (*P. communis*), Annapolis Valley/Nova Scotia, 2000, G. Braun | A (d) |
| EaCa4/97 | apple (*M. domestica*), Annapolis Valley/Nova Scotia, 1997, G. Braun | B/Pt4 (a) |
| EaCa6/97 | apple, Annapolis Valley/Nova Scotia, 1997, G. Braun | B/Pt4 (a) |
| EaCaH6 | Harrow, D. Hunter | B/Pt4 |
| EaCaH9 | Harrow, D. Hunter | Pt1 |
| EaCaL4 | London, D. Hunter | B/Pt4 |
| EaCaS16 | Simcoe, D. Hunter | Pt1 |
| EaCaS23 | Simcoe, D. Hunter | Pt1 |
| EaCaS5 | Simcoe, D. Hunter | Pt1 |
| EaCaV15 | Niagara, D. Hunter | By |
| EaCaV18 | Niagara, D. Hunter | Bz |
| EaCaV8 | Niagara, D. Hunter | Bx |
| EaCaW2E | Wentowth country/Hamilton, D. Hunter | Pt1 |
| EaCaW3 | Wentowth country/Hamilton, D. Hunter | B/Pt4 |
| EaKa6/00 | apple (*M. domestica*), Kentville, this work | B/Pt4 |
| EaKa7/00 | apple, Kentville/Nova Scotia, 2000, this work | B/Pt4 |
| EaKa8/00 | apple, Kentville/Nova Scotia, 2000, this work | – |
| EaKa9/00 | apple, Kentville/Nova Scotia, 2000, this work | – |
| EaKa10/00 | apple, Kentville/Nova Scotia, 2000, this work | – |
| EaKh14/00 | hawthorn (*Crataegus* sp.), Kentville/Nova Scotia, 2000, this work | – |
| EaKh15/00 | hawthorn (*Crataegus* sp.), Kentville/Nova Scotia, 2000, this work | B/Pt4 |
| EaKh17/00 | hawthorn, Kentville/Nova Scotia, 2000, this work | B/Pt4 |
| EaKp1/00 | pear (*P. communis*), Kentville/Nova Scotia, 2000, this work | A |
| EaKp2/00 | pear (*P. communis*), Kentville/Nova Scotia, 2000, this work | A |
| EaKp5/00 | pear (*P. communis*), Kentville/Nova Scotia, 2000, this work | – |
| EaTp3/00 | pear (*P. communis*), Niagara Falls/Ontario, 2000, this work | C |
| EaTp7/00 | pear (*P. communis*), Niagara Falls/Ontario, 2000, this work | D |
| EaTp9/00 | pear (*P. communis*), Niagara Falls/Ontario, 2000, this work | – |
| EaTp10/00 | pear (*P. communis*), Niagara Falls/Ontario, 2000, this work | – |
| EaTp12/00 | pear (*P. communis*), Niagara Falls/Ontario, 2000, this work | – |
| EaTpyr6/00 | Asian pear (*P. pyrifolia*), Niagara Falls/Ontario, 2000, this work | – |
| *E. amylovora* strains from USA (fruit tree) | | |
| CA1R | apple, California, A. Jones | E |
| CA263 | apple or pear, California, A. Jones | E |
| CA3R | apple, California, A. Jones | F |
| Ea88 | pear, Washington, A. Jones | E |
| Ea110 | apple, Michigan, A. Jones | B/Pt4 |
| Ea153 | USA, L. Pusey | Pt1 |
| EaU8/96 | apple, Utah, 1996 (Bereswill *et al.*, 1998) | – |
| EL01 | A. Jones | B/Pt4 |
| FB93-5 | pear, Idaho, A. Jones | E |
| IH3-1 | Indian hawthorn, Louisiana, A. Jones | G |
| IL1196 | pear, Washington, A. Jones | E |
| LA029 | pear, Washington, A. Jones | E |
| LA033 | pear, Washington, A. Jones | E |
| LP100 | apple, Washington, A. Jones | E |
| OR1 | pear, Oregon, A. Jones | E |
| OR6 | pear, Oregon, A. Jones | E |
| UTRJ2 | apple, Utah, A. Jones | B/Pt4 |
| WSDA14 | apple, Washington, A. Jones | B/Pt4 |
| WSDA34 | apple, Washington, A. Jones | E |
| *E. amylovora* strains from raspberry (isolated in North America) | | |
| EaCa1/95 | raspberry (*Rubus ideus*), Annapolis Valley, Nova Scotia | – (b) |
| EaCa1/98 | raspberry, Bouctouche, New Brunswick | – (bx) |
| EaCa8/96 | raspberry, Bouctouche, New Brunswick | – (c) |
| EaMR1 | raspberry, Michigan | K |
| EaRKK3 | raspberry, Michigan | J |
| EaRUB7 | raspberry (Bereswill *et al.*, 1998) | I |
| IL6 | raspberry, Illinois | H (e) |
| *E. amylovora* strains from Europe (Jock *et al.*, 2002) | | |
| CFBP1430 | *Crataegus* sp., France, J.-P. Paulin | Pt3a |
| Ea1/79 | *Cotoneaster* sp., Germany, 1979 | Pt1 (a) |
| Ea9–7 | *P. communis*, Toulouse (France), 1994 | Pt4 |
| Ea296 | *C. salicifolius*, Austria, 1993, M. Keck | Pt1 |
| Ea321 | CFBP1367, *Crataegus* sp., France, via S. Beer | Pt3 |
| EaFR3/98 | *Cotoneaster*, sp., Freiburg (Germany) | Pt1s |

**Table 1.** *Cont.*

| Strain | Description of isolation (plant, place, year, provider) | PFGE pattern[a] |
|---|---|---|
| EaUK2/98 | hawthorn, Kent (UK), 1998 | Pt1 |
| P1573 | *Cotoneaster* sp., Dorset (UK), 1995, A. Aspin | Pt4 |
| *E. pyrifoliae* strains from Korea (Kim *et al.*, 2001b) | | |
| Ep1/96 | Asian pear (*Pyrus pyrifolia*), South Korea, 1996 | PtA |
| Ep4/97 | Asian pear (*P. pyrifolia*) South Korea, 1996 | PtB |
| Ep28/96 | Asian pear (*P. pyrifolia*), South Korea, 1996 | PtC |
| Ep31/96 | Asian pear (*P. pyrifolia*), South Korea, 1996 | PtC |
| Ep102/98 | Asian pear (*P. pyrifolia*), South Korea, 1998 | PtA |
| Erwinia strains from Japan (Kim *et al.*, 2001a) | | |
| Ejp546 | Asian pear (*P. pyrifolia*), Hokkaido, 1979, A. Tanii | other |
| Ejp547[b] | Asian pear (*P. pyrifolia*), Hokkaido, 1979, A. Tanii | PtJp1 |
| Ejp556 | Asian pear (*P. pyrifolia*), Hokkaido, 1994, A. Tanii | other |
| Ejp557 | Asian pear (*P. pyrifolia*), Hokkaido, 1994, A. Tanii | PtJp1 |
| Ejp562 | Asian pear (*P. pyrifolia*), Hokkaido, 1994, A. Tanii | PtJp1 |
| Ejp617 | Asian pear (*P. pyrifolia*), Hokkaido, 1996, R. Roberts | other |

**a.** Letters A to K refer to the pattern of *Xba*I digests, as for Pt1 to Pt4 and PtJp1; highly related pattern are listed with '/', similar patterns with a lower case letter added to the main type in upper case. (a to e) in this column refer to *Spe*I-digests of genomic DNA as for PtA, PtB and PtC of *E. pyrifoliae*. -, not assayed.
**b.** Previously named Ejp546a, derived from a culture obtained with Ejp546.

from a narrow area in Nova Scotia were not identical in SSRs displaying numbers of 5, 7, or 9 (Fig. 3, Table 2). These data suggest independent changes of *E. amylovora* populations for SSR. In particular, a strain (EaTp12/00) isolated from a pear tree in the neighbourhood of the orchard, where other strains listed in Table 2, had been isolated, showed a divergent SSR number.

### *Sequence analysis of the* hrpN *genes of* E. amylovora *'fruit tree' and raspberry strains*

The *hrpN* genes from several *E. amylovora* 'fruit tree' strains with divergent PFGE patterns and from three raspberry strains were cloned by PCR amplification. The European 'fruit tree' strains Ea1/79, CFBP1430, Ea321 (nucleotide sequence from data library), Ea9–3, P1573 or EaFR3/97 with pattern Pt1, Pt3 (2x), Pt4 or Pt1A, respectively, showed almost identical nucleotide sequences for their *hrpN* genes with differences of not more than one nucleotide. On the other hand, the American raspberry strains could be distinguished by their HrpN sequences from 'fruit tree' strains from North America. Three motifs in the N-terminal part are typical for rubus strains and can even be considered diagnostic for their distinction from 'fruit tree' strains (Fig. 3A, boxes). In addition, the rubus strain EaCA1/95 showed a six amino acid insertion sequence in the centre of HrpN and a smaller insertion closer to the N-terminus. These sequences distinguished strain EaCA1/95 from strains EaMR1 and IL6 (Fig. 3A, underlined). In a dendrogram, the 'fruit tree' strain Ea1/79 from Germany is well separated from the aligned American rubus strains, but all *E. amylovora* strains differ in their alignment patterns from the Asian pear pathogens (Fig. 3B).

**Table 2.** SSR numbers of *E. amylovora* strains isolated 2000 in Eastern Canada.

| Origin | Isolated from | Name | SSR |
|---|---|---|---|
| Kentville | pear | EaKp1/00 | 7 |
| | | EaKp2/00 | 7 |
| | | EaKp5/00 | 7 |
| | apple | EaKa6/00 | 9 |
| | | EaKa7/00 | 9 |
| | | EaKa8/00 | 8 |
| | | EaKa9/00 | 7 |
| | | EaKa10/00 | 5 |
| | hawthorn | EaKh14/00 | >10 |
| | | EaKh15/00 | 8 |
| | | EaKh17/00 | 8 |
| Toronto | *P. pyrifolia* | EaTpyr6/00 | 4 |
| | pear | EaTp9/00 | 4 |
| | | EaTp10/00 | 4 |
| | pear[a] | EaTp12/00 | 3 |

**a.** From tree adjacent to main orchard.

### *Sequence analysis of the* hrpN *genes of* E. pyrifoliae *strains and Erwinia strains from Japan*

*Erwinia amylovora* 'fruit tree' and raspberry strains share motifs of HrpN with the Asian pear pathogens. In Fig. 3A, the sequences of the Korean *Erwinia pyrifoliae* Ep1/96 and of an *Erwinia* strain from Japan, Ejp557, were aligned for their possible relationship to the *E. amylovora* raspberry strains. *Erwinia pyrifoliae* strains and the *Erwinia* strains from Japan were strikingly distinct from both *E.*

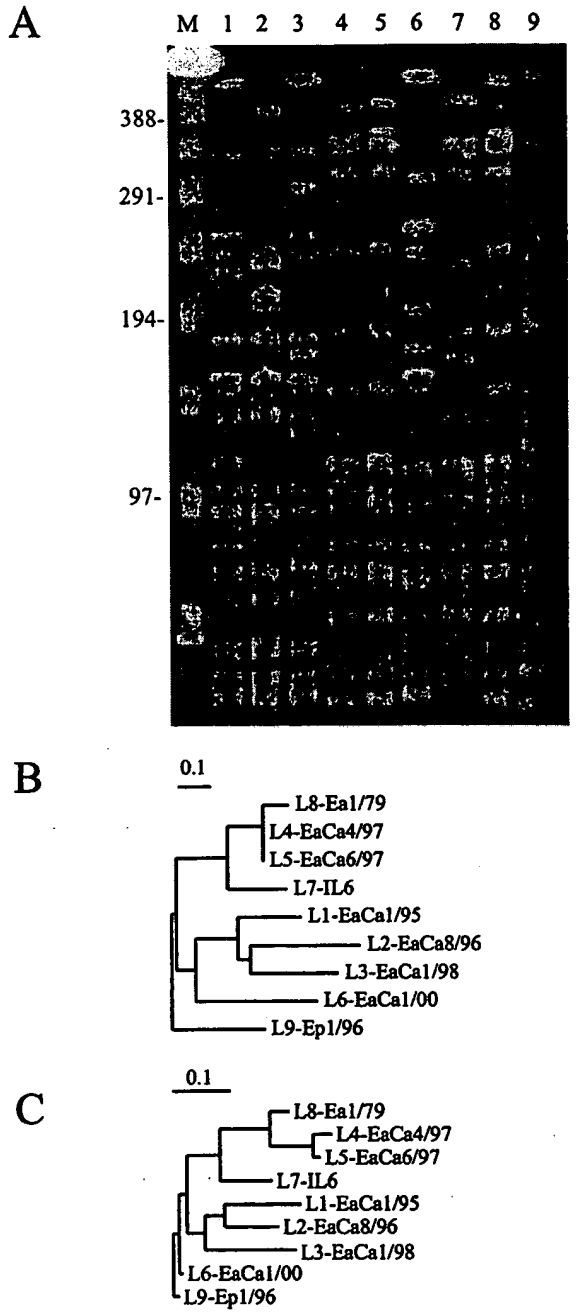


**Fig. 2.** PFGE analysis of *E. amylovora* strains isolated from raspberry in Canada and Illinois by genomic *Spe*I digests in comparison with isolates from apple in Canada and an *E. amylovora* isolate from cotoneaster and an *E. pyrifoliae* strain.
A. Lanes: M: λ DNA marker (sizes at left in kb); 1: EaCa1/95 (rb); 2: EaCa8/96 (rb); 3: EaCa1/98 (rb); 4: EaCa4/97 (a); 5: EaCa6/97 (a); 6: EaCa1/00 (p); 7: IL6 (rb); 8: Ea1/79 (highest band from partial digest); 9: Ep1/96 (*E. pyrifoliae*).
B. Dendrogram from pattern in A.
C. Dendrogram from pattern of *Xba*I digest with the strains applied in A. Suffix 'a', isolated from apple; 'p', from pear; 'IL6', from raspberry. Bars, distance scales.

*amylovora* groups. The HrpN sequences of the two Asian pear pathogens were related to each other, but not identical and differed in at least four clusters of more than two amino acids.

The *E. pyrifoliae* strains Ep1/96 and Ep102/98 belong to the PFGE pattern type PtA, Ep4/97 to PtB and Ep28/96, Ep31/96 to pattern type PtC (Kim *et al.*, 2001b). Most parts of their HrpN sequences were identical. Nevertheless, Ep1/96, Ep4/97 and Ep102/98 showed a DNA insertion encoding seven amino acids, which distinguished them from the others (Fig. 4). The motif 'GGSGGGL' is reiterated twice for these strains, but is not conserved for *E. amylovora* or the *Erwinia* strains from Japan (Fig. 3A and Fig. 4A). The distance scale in the dendrogram derived in Fig. 4B indicates a close relationship of the investigated *E. pyrifoliae* strains with small differences. Ep1/96, Ep28/96 and Ep102/98 are highly related, less Ep31/96, whereas Ep4/97 is more distinct from the others.

The *Erwinia* strains from Japan analysed were also not completely homogenous for their HrpN sequences. Strains Ejp547, Ejp557 and Ejp562 were highly related in the PFGE patterns after *Xba*I digests, whereas the others could be separated from the first group on this basis (Kim *et al.*, 2001a). In agreement with those data, the HrpN proteins of Ejp547, Ejp557 and Ejp562 differed from the amino acid sequences derived from the other strains at five sites of HrpN (Fig. 5A). The dendrogram in Fig. 5B indicates the relationship of Ejp557, Ejp547 and Ejp562, separating them from the other strains, also confirming PFGE data that strain Ejp547 is not identical with strain Ejp546, obtained in the same agar culture.

The sequences of the *hrpN* genes of *E. amylovora* 'fruit tree' and raspberry strains as well as of *E. pyrifoliae* strains and *Erwinia* strains from Japan showed a differential degree of conservation. The *E. amylovora* 'fruit tree' and rubus strains were 97% related to each other, whereas the HrpN proteins of *E. pyrifoliae* and *Erwinia* strains from Japan had only 83% similarity to HrpN of *E. amylovora* 'fruit tree' strains.

A summarizing dendrogram (Fig. 6) grouped the *E. amylovora* strains apart from the two Asian pear pathogens. *E. pyrifoliae* strains are highly related to each other, and less to the *Erwinia* strains from Japan.

## Discussion

The PFGE patterns of the strains isolated in North America are divergent, in contrast to the pattern of strains from Central Europe and the Mediterranean region, which were grouped into four main pattern types (Jock *et al.*, 2002). In spite of basically unrestricted trade in fruit and fire blight host plants, there has been no obvious mixing of pattern types in Europe and the Mediterranean region. Sequential spread from infected sites is the dominant way of disease

A

```
Ea1/79      (1) mslntsglgastmqisiggaggnngllgtsrqnaglggnsalglgggnqndtvnqlaglltgmmmmmsmmggggl---mg
IL6         (1) ..........................................................................---..
EaMR1       (1) ..........................................................................---..
EaCa1/95    (1) ..........................................................................---..
Ep1/96      (1) ......a.............g.............dh.........n.........m.................tgll.
Ejp557      (1) ......a.............g.............dh.........n.........m.................---t.

Ea1/79     (78) ----gglgggl-----gngl--g-gsgg-----------lgeglsnalndmlggsln-----tlgskggnnttsttnsp
IL6        (78) lmg-..............------.-----------.....f...q-----.....................
EaMR1      (78) lmg-..............------.-----------.....f...q-----.....................
EaCa1/95   (78) lmg-.............g..--.n.l..----------.....f...q-----.....................
Ep1/96     (81) -----..f....lgggs.g..--.-....glgglgghlgst..g.igggigga...p.gatvgts...gi.gsaa.gvg.a
Ejp557     (78) llgg......f-----.g..lg.-....glgglggdlgst..g..gagig.a...p.gatvgts...gi.gsaa.gvg.a

Ea1/79    (129) ldqalginstsqnd------dst------sgtdstsdssdpmqqllkmfseimqslfgdgqdgtqgsssggkqptegeqn
IL6       (129) ..................------.-------.....................................................
EaMR1     (129) ..................------..------.....................t...............................
EaCa1/95  (133) ..................-------...fgtdst..................................................
Ep1/96    (154) ..................------s..-------.....s......v...m...........e......sg..a.........s
Ejp557    (152) ..............sstsgt...------.....s......v...m...........e......sg..a.........s

Ea1/79    (197) aykkgvtdalsglmgnglsqllgnggllgggqggnagtgldgsslggkglqnlsgpvdyqqlgnavgtgigmkagiqalnd
IL6       (197) ..........................................................................
EaMR1     (197) ..........................................................................
EaCa1/95  (207) ....................f.....................................................
Ep1/96    (222) ......s....a........t.............s........g.....................................
Ejp557    (226) ......s....a........t.......a....s........g.....................................

Ea1/79    (277) igthsdsstrsfvnkgdramakeigqfmdqypevfgkpqyqkgpgqevktddkswakalskpdddgmtpasmeqfnkakg
IL6       (277) ..........................................................................
EaMR1     (277) ..........................................................................
EaCa1/95  (287) .............d............................................................
Ep1/96    (302) ..........................................................................
Ejp557    (306) ..........................................................................

Ea1/79    (357) miksamagdtgngnlqargaggsslgidammagdainnmalgk
IL6       (357) ..................h.......................
EaMR1     (357) ..........................................
EaCa1/95  (367) ..........................................
Ep1/96    (382) ..................................t.......
Ejp557    (386) ..................................t.......
```

B

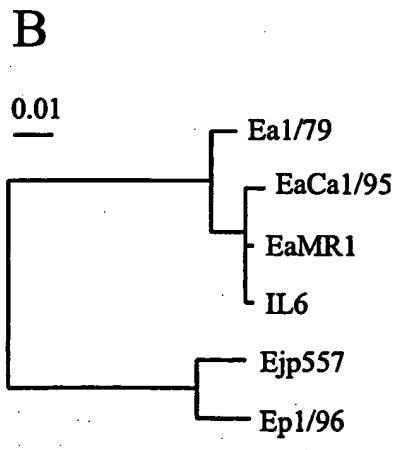


**Fig. 3.** Sequence alignment of the HrpN proteins from raspberry strains isolated in Canada compared with the *E. amylovora* 'fruit tree strain' Ea1/79, *E. pyrifoliae* Ep1/96 and Ejp557, an *Erwinia* strain from Japan.
A. Common motifs for raspberry strains are boxed and unique insertions for strains are underlined.
B. Dendrogram from the amino acid sequences aligned in A. Bar, distance scale.

A

```
Ep1/96     (1) mslntsalgastmqisiggagggnglltgtsrqnaglgdhsalglgggnnndtvnqlagmltgmmmmmsmmgggltgllg
Ep4/97     (1) ................................................................................
Ep28/96    (1) ................................................................................
Ep31/96    (1) ................................................................................
Ep102/98   (1) ................................................................................

Ep1/96    (81) ggfgggllggggsgggggsggglgglgghlgstlgggigggiggalggplgatvgtslgsgiggsaasgvgsaldqalgi
Ep4/97    (81) ..........|.....|...s.....d.....|.....|.....|.....|.....|.....|.....|.....|
Ep28/96   (81) ..........|------|.....d.....|.....|.....|.....|.....|.....|.....|.....|
Ep31/96   (81) ..........|------|.....v...d.....|.....|.....|.....|.....|.....|.....|.....|
Ep102/98  (81) ..........|.....|.....d.....|.....|.....|.....|.....|.....|.....|.....|

Ep1/96    (161) nstsqndsstsgtdsssdsedpvqqlmkmfseimqslfgegqdgtqsgssagkqptegeqsaykkgvsdalsalmgngls
Ep4/97    (161) ................................................................................
Ep28/96   (154) ................................................................................
Ep31/96   (154) ................................................................................
Ep102/98  (161) ................................................................................

Ep1/96    (241) qtlgngglgggqggsagtgldgsglggkglqnlsgpvdyqqlgnavgtgigmkagiqalndigthsdsstrsfvnkgdra
Ep4/97    (241) ................................................................................
Ep28/96   (234) ................................................................................
Ep31/96   (234) ................................................................................
Ep102/98  (241) ................................................................................

Ep1/96    (321) makeigqfmdqypevfgkpqyqkgpgqevktddkswakalskpdddgmtpasmeqfnkakgmiksamagdtgngnlqarg
Ep4/97    (321) ..................................e.............................................
Ep28/96   (314) ................................................................................
Ep31/96   (314) ................................................................................
Ep102/98  (321) ................................................................................

Ep1/96    (401) aggsslgidammagdtinnmalgk
Ep4/97    (401) ........................
Ep28/96   (394) ........................
Ep31/96   (394) ...........r............
Ep102/98  (401) ........................
```

B

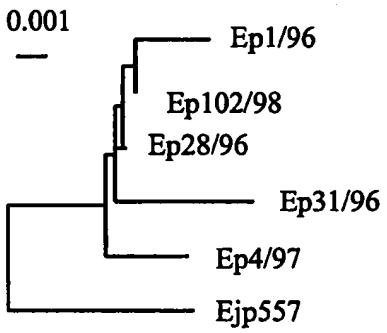


**Fig. 4.** Comparison of HrpN proteins from five *E. pyrifolia* strains.
A. Amino acid aligment. The motif for strain differentiation is boxed.
B. Dendrogram from the amino acid sequences aligned in A. Bar, distance scale.

distribution, except for introduction of fire blight into Central Spain and Northern Italy, where plant imports can be connected with appearance of fire blight caused by *E. amylovora* strains displaying pattern type Pt3, which has not been found in the adjacent regions.

An ordered PFGE pattern was not found for strains from North America, because even a relatively low number of isolates gave rise to several different patterns. They also differ from European patterns except for Pt1 and Pt4, which were found repeatedly in isolates from Eastern Canada. Thus, Pt1 and Pt4 could have originated in North America and were then distributed to Europe (Jock *et al.*, 2002), first to England with the first European fire blight outbreaks (Billing and Berrie, 2002). The other patterns in

A

```
Ejp546   (1) mslntsalgastmqisiggagggnglltgtsrqnaglgdhsalglgggnnndtvnqlagmltgmmmmmsmmgggltgllg
Ejp547   (1) ...............................................................................
Ejp556   (1) ...............................................................................
Ejp557   (1) ...............................................................................
Ejp562   (1) ...............................................................................
Ejp617   (1) ...............................................................................

Ejp546  (81) ggglgggfgggllgggsgggglgglggdlgstlggglgagiggalggplgatvgnslgssiggsaasgagsaldqalgins
Ejp547  (81) ..............................................................t....g........v.....
Ejp556  (81) ...............................................................................
Ejp557  (81) .........................................d.....t....g........v.....
Ejp562  (81) ..............................................................t....g........v.....
Ejp617  (81) ...............................................................................

Ejp546 (161) tsqmdsstsgtds------ssdssdpvqqlmkmfseimqslfgegqdgtqsgssagkqptegeqsaykkgvsdalsalmg
Ejp547 (161) ............tsgtds...........................................................
Ejp556 (161) ............------...........................................................
Ejp557 (161) ............tsgtds...........................................................
Ejp562 (161) ............tsgtds...........................................................
Ejp617 (161) ............------...........................................................

Ejp546 (235) nglsqtlgnggl gggqggsagtgldgsglggkglqnlsgpvdfqqlgnavgtgigmkagiqalndigthsdsstrsfvnk
Ejp547  (241 .....................................y.....................................n.....
Ejp556 (235) ...............................................................................
Ejp557 (241) ..........a.....................y.....................................
Ejp562 (241) .....................................y.....................................
Ejp617 (235) ................s..............................................................

Ejp546 (315) gdramakeigqfmdqypevfgepqyqkgpgqevktddkswakalskpdddgmtpasmeqfnkakgmiksamagdtgngnl
Ejp547 (321) .ep..............k................................................................
Ejp556 (315) ..............k................................................................
Ejp557 (321) ..............k................................................................
Ejp562 (321) ..............k................................................................
Ejp617 (315) ..............k.....k..............................................................

Ejp546 (395) qargaggsslgidammagdtinnmalgk
Ejp547 (401) ............................
Ejp556 (395) ............................
Ejp557 (401) ............................
Ejp562 (401) ............................
Ejp617 (395) ............................
```

B

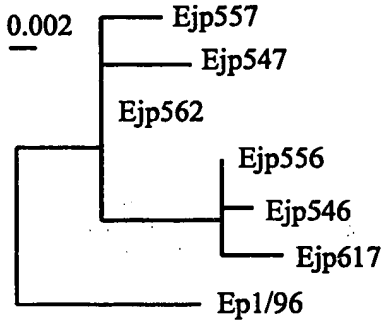


**Fig. 5.** Comparison of HrpN proteins from six *Erwinia* strains isolated in Japan.
A. Amino acid alignment. An insertion motif and amino acid substitutions for strains Ejp547, Ejp557, and Ejp562 are underlined.
B. Dendrogram from the amino acid sequences aligned in A. Bar, distance scale.

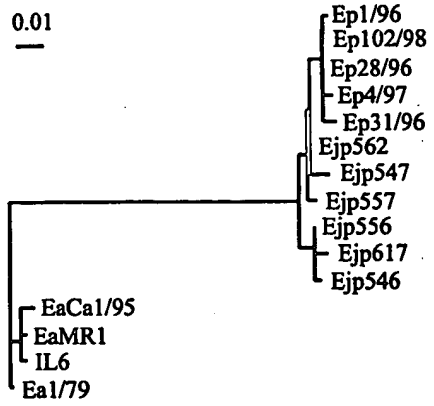


**Fig. 6.** A dendrogram showing the relatedness of *E. amylovora* 'fruit tree' and raspberry strains and the evolutionary distance of the Asian pear pathogens based on the HrpN amino acid sequences. Bar, distance scale.

America can be explained by genetic changes over a long time period; they were rarely distributed to other countries except for Pt2, a pattern which was found first in Egypt and also in a strain from California (Zhang and Geider, 1997; Jock *et al.*, 2002).

A special subgroup of *E. amylovora* strains from raspberry is endemic in North America and has only been isolated there. A reason for the difference in the PFGE patterns from *E. amylovora* 'fruit tree' strains could be the unusual host, which required many genomic changes for adaptation of the pathogen. On the other hand, their presumably long persistence in North America could have allowed accumulation of many base changes in the genome causing their pattern heterogeneity.

The SSR numbers are not related to PFGE patterns or the areas of isolation as also found for American (Schnabel and Jones, 1998) and European *E. amylovora* strains (Kim and Geider, 1999). Nevertheless, different numbers indicate non-identical isolates from fire blighted plants. Among intermediate numbers there is a high occurrence of low numbers such as 3 and 4, which are not often observed in Central Europe. In isolations from the same apple orchard in Kentville, we found SSR numbers from 5, 7 and 9. Normally, only one SSR-type is usually isolated in the same set of isolates, but recently, we observed some exceptions like in England where we found SSR type 3 and 7 in isolates from adjacent plants (Jock *et al.*, 2003a).

The ability to induce a hypersensitive response (HR) on non-host plants is a common feature of plant pathogenic bacteria. In evolution, many genes of the *hrp* cluster especially those involved in protein secretion have been highly conserved among bacteria (Van Gijsegem *et al.*, 1993; Bogdanove *et al.*, 1996). A spontaneous base change in *hrpL* within an *E. pyrifoliae* population has been recently described (Jock *et al.*, 2003b). Genes encoding harpins are highly divergent even for related bacteria. The HrpN proteins of two related species such as *P. stewartii* ssp. *stewartii* (*E. stewartii*) and *P. stewartii* pv. gypsophylae show only 60% similarity to each other (EMBL Nucleotide Sequence Database accession numbers AF282857 and AF21176 respectively). The similarity of these harpins and HrpN of *E. carotovora* ssp. *carotovora* (AF302656) to harpin of *E. amylovora* is 62%, 56%, and 49% respectively. The sequence information of *hrpN* is not only suited for classification of bacterial species, but also for grouping of strains within a species. On the other hand, HrpN can be conserved, found for *E. amylovora* 'fruit tree' strains, where the sequences matched at the nucleotide level. These strains isolated from raspberry in North America, share more than 95% similarity. A high relationship was also observed between *E. pyrifoliae* strains from Korea and the Japanese pear pathogen, whereas *E. amylovora* strains match with these pathogens less than 85%. Although the *Erwinia* stains from Japan have not been taxonomically classified, the relatedness of HrpN proteins adds to the notion to place these with *E. pyrifoliae* into the same species (Kim *et al.*, 2001a). In addition, HrpN sequences provided also information for strain differentiation within a species.

Because the transport of harpin depends on several cellular proteins, its sequence cannot freely change only to conserve its elicitor activity. Whether the HrpN protein or even the DspA/E-protein (Gaudriault *et al.*, 1997; Bogdanove *et al.*, 1998) contribute to host plant specificity of a pathogen has still to be shown. The divergences of the HrpN sequences should indicate an evolutionary drift, similar to the PFGE patterns analysed. The most likely explanation is the long persistence of *E. amylovora* in North America, of *E. pyrifoliae* in Korea and the slightly different pear pathogen in Japan. Furthermore, the occurrence of European pattern types Pt1 and Pt4 among the divergent American PFGE patterns may indicate a rare escape of fire blight from its origin in North America.

## Experimental procedures

### *Bacterial strains, PCR and PFGE analyses*

The *E. amylovora* strains were isolated in the Eastern part of Canada, or were gifts from colleagues (Table 1). They were confirmed as *E. amylovora* on several agar plates including MM2Cu (Bereswill *et al.*, 1998) and by PCR assays (Bereswill *et al.*, 1992). Pulsed-field gel electrophoresis analysis (Jock *et al.*, 2002) and determination of the SSR numbers (Kim *et al.*, 1999) were done as described. *Erwinia pyrifoliae* (Kim *et al.*, 2001b) and the *Erwinia* strains from Japan were also described previously (Kim *et al.*, 2001a). For pattern comparison, the PFGE fragments were assigned by eye with letters and the program CLUSTALX1.81 used for pairwise alignments. The dendrograms were adjusted with NJ-tree and further processed in a graphics program. Pattern analy-

sis was also done with the public domain programs ImageJ (v. 1.30; W. Rasband, NIH, USA) and Cross Checker (v. 2.91; J. B. Buntjer, Wageningen, the Netherlands) and alignment with Treecon vs. 1.3b (Y. van de Peer, Konstanz, Germany) and CLUSTALX1.81 respectively. Corrections by eye were required for further adjustment of the band assignments.

### *Analysis of the* hrpN *genes from* E. amylovora *and the Asian pear pathogens*

The *hrpN* genes of strains from the three pathogens were amplified with PCR consensus primers, which were deduced by comparison of several known nucleotide sequences from plant pathogens namely *E. amylovora* (EMBL Nucleotide Sequence Database accession number M92994) or *P. stewartii* (accession number AF282857). Primer HRPN1 was 5′-ATGAGTCTGAATACAAG-3′ (at start of *E. amylovora hrpN*) and primer HRPN3c 5′-GCTTGCCAAGTGCCATA-3′ (in *hrpN*, 11 bp downstream from stop codon). In some cases, weak PCR bands obtained could indicate incomplete matching of the primers. The amplified DNA fragments were cloned into vector pGEM-T and were commercially sequenced. To cover the total *hrpN* genes, a third primer HRPMc (5′-CCACGGCGTTACCCAACTGCTGG-3′) located in the central part of the *hrpN* gene was used to cover gaps in the HrpN sequences. Alignments and dendrograms were created with CLUSTALX1.81.

*Erwinia pyrifoliae* and the *Erwinia* strains from Japan were considered to be sufficiently related to *E. amylovora* to amplify their *hrpN* genes with the *Erwinia* PCR consensus primers given above. This was indeed possible and allowed cloning and sequencing of their *hrpN* DNA fragments as for *E. amylovora* by using primers HRPN1 and HrpN3c. A primer comprising the stop codon at the C-terminus of *hrpN* did not result in the formation of a PCR product together with primer HRPN1.

The *hrpN* nucleotide sequences from strains Ea1/79, EaCa1/95, IL6, EaMR1, Ejp546, Ejp557, Ep1/96, Ep31/96, Ep4/97 and were deposited in the EMBL Nucleotide Sequence Database with the accession numbers AJ579689 (Ea1/79), AJ579690 (EaCa1/95), AJ579691 (IL6), AJ579692 (EaMR1), AJ579693 (Ejp546), AJ579694 (Ejp557), AJ579695 (Ep1/96), AJ579696 (Ep31/96) and AJ579697 (Ep4/97).

## Acknowledgements

Samples of necrotic plants from orchards with fire blight were collected by us in Canada with the help of Gordon Braun, Paul Hildebrand (Kentville/Nova Scotia), Martin Hubbes and David Hunter (Toronto/Ontario). We also thank Al Jones (East Lansing/Michigan), David Hunter and Gordon Braun for sending additional *E. amylovora* strains as well as Carlo Bazzi (Bologna) and Christopher Hayward (Brisbane) for comments on the manuscript.

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